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(21) International Application Number: PCT/EP98/01914 (22) International Filing Date: 24 March 1998 (24.03.98) (30) Priority Data: 97201022.7 7 April 1997 (07.04.97) EP (34) Countries for which the regional or international application was filed: NL et al. 97204062.0 22 December 1997 (22.12.97) EP (34) Countries for which the regional or international application was filed: NL et al. (71) Applicant (for all designated States except AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG US): UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL). (71) Applicant (for AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG only): UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): BEIJERSBERGEN, Alida, Godelieve, M. [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). BUNDOCK, Paul [NL/NL]; Rijks Universiteit Leiden, Instituut Moleculaire Plantkunde, Clusius Laboratorium, Wasse-		naarseweg 64, NL-2333 AL Leiden (NL). GOUKA, Robertus, Johannes [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). DE GROOT, Marcellus, Johannes, A. [NL/NL]; Unilever Research Vlaardingen, Olivier van Noortlaan 120, NL-3133 AT Vlaardingen (NL). HOOYKAAS, Paul, Jan, J. [NL/NL]; Rijks Universiteit Leiden, Instituut Moleculaire Plantkunde, Clusius Laboratorium, Waskenaarseweg 64, NL-2333 AL Leiden (NL). (74) Common Representative: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL). (81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
(54) Title: AGROBACTERIUM MEDIATED TRANSFORMATION OF MOULDS, IN PARTICULAR THOSE BELONGING TO THE GENUS ASPERGILLUS (57) Abstract <p>The invention relates to <i>Agrobacterium</i> mediated transformation of moulds comprising species of the fungal sub-divisions <i>Ascomycotina</i>, <i>Basidiomycotina</i>, <i>Deuteromycotina</i>, <i>Mastigomycotina</i>, and <i>Zygomycotina</i>. Examples demonstrate the transformation of <i>Aspergillus awamori</i> (both protoplasts and conidia), <i>Aspergillus nidulans</i>, <i>Aspergillus niger</i>, <i>Colletotrichum gloeosporioides</i>, <i>Fusarium solani pisi</i>, <i>Neurospora Crassa</i>, <i>Trichoderma reesei</i>, <i>Pleurotus ostreatus</i> and <i>Agaricus bisporus</i> (all conidia), and <i>Fusarium graminearum</i> (both conidia and rehydrated freeze dried ATCC material). Especially for <i>Aspergillus awamori</i> the transformation frequency is much higher than with conventional mould transformation techniques. It has further been found that not only one expressable gene can be introduced into these moulds, but even multiple copies of such gene, which, moreover, can be targeted e.g. in the chromosomal <i>pyrG</i> locus, as exemplified for <i>A. awamori</i>. These multiple copies can be of a gene encoding a desired, homologous or heterologous, protein.</p>		